PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

19/C
B. Webb
3/30/01

In re application of

Takahiko, ISHIGURO, et al.

CPA of
Appln. No.: 09/345,761

Confirmation No. Not Yet Assigned

Group Art Unit: 1655

Filed: March 21, 2001

Examiner: WILDER, C

For: METHOD OF ASSAY OF TARGET NUCLEIC ACID

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE CLAIMS:

Please cancel claims 1-20 and 29 .without prejudice or disclaimer.

Please add the following new claims 30-50:

30 A method for assaying a single-stranded RNA in a sample, wherein said RNA contains a specific nucleic acid sequence, said method comprising the following steps:

(1) providing a single-stranded RNA comprising said specific nucleic acid sequence;

(2) exposing said first sequence to a reagent (A), which allows the single-stranded RNA to be cut at the 5' end of the specific nucleic acid sequence;

(3) cutting the single-stranded RNA at the 5' end of the specific nucleic acid sequence;

(4) hybridizing to said product of step (3), a reagent (B), which is a short single-stranded oligo DNA primer complementary to a sequence at the 3' end of said specific nucleic acid sequence;

C1

Sub D1

(5) extending said first single-stranded oligo DNA primer to the 5' end of the specific nucleic acid sequence with a reagent (C), which is an RNA-dependent DNA polymerase and with a reagent (E), which is deoxynucleoside triphosphates, to form a DNA-RNA double-strand;

Sub D1

(6) digesting the RNA strand of said DNA-RNA double-strand from step (5) with a reagent (D), which is a ribonuclease that degrades RNA in a DNA-RNA double-strand to give a single-stranded DNA complementary to said specific nucleic acid sequence;

(7) hybridizing to said single-stranded DNA from step (6) a reagent which is a second single-stranded oligo DNA primer having the following sequences, in the following order, beginning at the 5' end and proceeding in a 5' to 3' direction: i) a promoter sequence for a DNA-dependent RNA polymerase, ii) an enhancer sequence for said promoter sequence, and iii) a sequence at the 5' end of said specific nucleic acid sequence;

C1 cont

(8) extending said second oligo DNA primer to the 5' end of said single-stranded DNA with a reagent (G), which is a DNA-dependent DNA polymerase and with said reagent (E);

(9) synthesizing a single-stranded RNA from said promoter sequence with a reagent (H), which is a DNA-dependent RNA polymerase and a reagent (I) which is ribonucleoside triphosphates;

(10) either:

(a) cycling said single-stranded RNA from step (9) to step (4), or

(b) hybridizing to said single-stranded RNA from step (9) a reagent (J), which is a single-stranded oligo DNA complementary to said specific nucleic acid sequence, labeled so that it gives off a measurable fluorescent signal upon hybridization with a nucleic acid containing said specific nucleic acid sequence; and

(11) after addition of reagents (A) to (J), measuring at least once a fluorescent signal from said hybrid formed in step (10) (b);

wherein said reagents (A) to (J) are added to a reaction vessel one by one, in functional combinations, or all at once.

31. The method according to Claim 30, wherein the reagent (A) is a single-stranded oligo-nucleic acid complementary to a sequence 5' of, and adjacent to, the 5' end of said specific nucleic acid sequence.

32. The method according to Claim 31, wherein the reagent (A) is a DNA, and the method further comprises adding an RNaseH and deactivating the RNaseH by heating or by addition of an inhibitor prior to addition of the reagent (B).

Sub D2

33. The method according to Claim 32, wherein addition of the reagent (A) is followed by simultaneous addition of the reagents (B) to (I), and then by addition of the reagent (J).

34. The method according to Claim 32, wherein addition of the reagent (A) is followed by simultaneous addition of the reagents (B) to (J).

C1 Cont

35. The method according to Claim 30, wherein the oligo nucleic acid as the reagent (A) is a ribozyme or a DNA enzyme.

36. The method according to Claim 30, wherein the reagent (C), an RNA-dependent DNA polymerase, is also the reagent (D), a ribonuclease that degrades RNA in a DNA-RNA double strand.

37. The method according to Claim 30, wherein an enzyme having both an RNA-dependent DNA polymerase activity and a DNA-dependent DNA polymerase activity is used as both the reagents (C) and (G).

38. The method according to Claim 37, wherein the enzyme is avian myoblastoma virus polymerase.

Sub D3

39. The method according to Claim 30, wherein the first and second oligo DNA primers as the reagents (B) and (F) are used at concentrations of from 0.02 to 1 μM.

40. The method according to Claim 30, wherein the DNA-dependent RNA polymerase as the reagent (H) is at least one enzyme selected from the group consisting of phage SP6 polymerase, phage T3 polymerase, and phage T7 polymerase.

41. The method according to Claim 30, wherein the single-straned oligo DNA as the reagent (J) is a DNA which is linked to a fluorescent intercalative dye so that the fluorescent intercalative dye changes its fluorescence characteristic upon hybridization of the DNA with another nucleic acid by intercalating into the resulting double strand.

42. The method according to Claim 30, wherein the single-stranded oligo DNA as the reagent (J) is a DNA which has a 3' end sequence that is not complementary to the specific nucleic acid sequence or has a modified 3' end, and hybridizes to the nucleic acid of Claim 30 having said specific nucleic acid sequence.

43. The method according to Claim 41, wherein the single-stranded oligo DNA as the reagent (J) is a DNA which has a 3' end sequence that is not complementary to the specific nucleic acid sequence or has a modified 3' end, and hybridizes to the nucleic acid of Claim 30 having said specific nucleic acid sequence.

Sub D3

44. The method according to Claim 30, which further comprises a step of detecting or quantifying the single-stranded RNA in the sample based on the measured fluorescent signal or change in the measured fluorescent signal.

C1 concld

45. The method according to Claim 30, wherein all the reagents are chloride-free.

46. The method according to Claim 30, wherein prior to said step (10)(b)acetate is added as a reagent.

47. The method according to Claim 46, wherein the acetate is magnesium acetate at a concentration of from 5 to 20 mM or potassium acetate at a concentration of from 50 to 200 mM.

48. The method according to Claim 30, wherein prior to said step (10)(b) sorbitol is added as a reagent.

~~49. The method according to claim 30, wherein the oligo nucleic acid as the reagent (A) is DNA.~~

Sub D4

50. The method according to Claim 30, wherein the temperature is selected from the range of from 35 to 60°C.

REMARKS

The above-requested amendment, canceling claims 1-20 and 29 and replacing them with claims 30-50 is made in order to address the Examiner's position that the functions of the various reactants should be recited in the claims. Support for the functions of the reagents A-I is found in the specification from page 18, line 15 to page 26, line 6. No new matter is added.

In the Advisory Action dated February 13, 2001, the Examiner maintained a rejection under 35 U.S.C. § 103.

Specifically, in paragraph number 2, beginning at page 4 of the Advisory Action, the Examiner stated that Applicants' arguments in the Amendment filed July 10, 2000 regarding Davey et al were not sufficient to overcome the art rejections for the following reasons:

(1) Applicants' arguments emphasizing specific functions of the various reagents (A) through (J) carry little weight because the claims do not recite the functions of the reagents.

(2) In particular, Applicants' arguments concerning the special feature of the present invention, namely that the target RNA does not have to have a specific sequence at the 5' end, are not convincing because the claims do not recite that the first single-stranded oligo nucleic acid functions to facilitate cutting of the single-stranded RNA in a sample at the 5' end. As the claims are written, the reagent (A) could function as a template, as in Clark et al.

Applicants' argument that the Davey et al reference does not disclose anything about the detection of amplified RNA with a fluorescent intercalative dye-labeled DNA is not convincing

because the rejection is an obviousness rejection and that element, missing from Davey et al, is found in Ishiguro et al. The Examiner further states that one of ordinary skill in the art would have readily combined Ishiguro et al with Davey et al in light of the teaching in Ishiguro et al that performing an amplification process with a fluorescent DNA intercalative dye allows the quantitative detection of RNA over a wide dynamic range.

The claims have been amended to recite the functions of the reagents A to J. Accordingly the Examiner is requested to reconsider and remove the rejections and pass the case to issuance.

Any inquiry concerning this communication should be directed to the undersigned at the telephone number listed below.

Entry and consideration of this Amendment is respectfully requested.

Respectfully submitted,

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